May 27, 1975

Dr. James C. Wang
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Dear Jim:

I realize that I should have contacted you sooner to bring you up to date on our situation and I am sorry it has been so long. After some amount of trouble we finally got our cell lines straightened out and made DNA in good yield again. About the same time we learned that Marty Gellert was set up with a ligase preparation that was free of nuclease. Therefore, we decided to proceed and make the molecules we wished ourselves using his enzyme. We have just recently finally done that, having been busy in the meantime preparing and purifying some tritiated f1 etc. Tikvah Vogel prepared two molecules of negative superhelicity bracketing the superhelicity of the isolated SV40 DNA, as well as a molecule at about zero superhelicity. The experiments are consistent with the results we obtained on binding in the presence of ethidium bromide, as detailed in the enclosed preprint which is in press in the PNAS, to be out in July. That is, the more negative the superhelicity, the better the extent of binding.

Rereading my original letter to you I am embarrassed by the extent to which my ignorance about superhelices showed through since I spoke about preparing molecules of positive superhelicity. Happily, I have now educated myself.

Because the results with the negative molecules we prepared are consistent with the results obtained in the presence of ethidium, we now have more confidence that the latter data are meaningful. Thus, we are more confident that the preferential binding is indeed both to positive and negative superhelices.

In the communication in the JBC we mentioned that the labeled histone itself did not bind to the nitrocellulose filters. We know now that the conclusion was wrong and that we were misled by the fact that the histone was in fact sticking to the tubes. We have had a lot of trouble finding a way to avoid this in order to carry out experiments on the stoichiometry.

By including a high concentration of serum albumin (1 mg per ml), which does not interfere with the reaction, we can now account for everything. This has allowed us to determine that the complex we are measuring is not centrifuged down under the conditions usually used for histone-DNA complex formation, so we believe that the assay is a typical nitrocellulose filter assay.

Many thanks for your generous offer of help. We certainly appreciated it when it didn't seem likely we could do the job here.

Sincerely yours,

Maxine Singer
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